



New perspectives in oral peptide delivery

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Owing to their structural diversity, peptides are a unique source of innovative active ingredients. However, their development has been challenging because of their disadvantageous pharmacokinetic (PK) properties. Over the past decade, many attempts have been made to improve the oral bioavailability of peptide drugs. In this review, we highlight the most recent and promising techniques aimed at the improvement of the oral bioavailability of peptides. The most recent findings will influence future approaches of pharmaceutical companies in the development of new, more efficient, and safer orally delivered peptides.

Introduction

According to Verified Market Research, the global therapeutic peptides market was valued at US\$26.98 billion in 2019 and is projected to reach US\$51.24 billion by 2027, growing at a compound annual growth rate (CAGR) of 8.7% from 2020 to 2027 [1]. According to 'Allied Market Research' the oral protein- and peptide-based drug market accounted for \$ 643 million in 2016, and is anticipated to reach \$ 8'233 million by 2028, registering a CAGR of 11.7 % from 2022 to 2028 [2].

The top-selling peptide drug for metabolic diseases is liraglutide (Victoza®), a glucagon-like peptide (GLP-1). Popular peptide drugs, including leuprolide (Lupron®), goserelin (Zoladex®), the somatostatin analogs octreotide, and lanreotide, contributed to >US\$4 billion in sales. The growth in the industry will likely be fueled by the expected increasing incidence of metabolic disorders and cancers. Clinical trials with oral protein and peptide products have progressively increased, culminating in September 2019, with the US Food and Drug Administration (FDA) approval of semaglutide (Rybelsus®) developed Novo Nordisk AG as an oral application against type 2 diabetes mellitus (T2DM). Drugs for oral use are dominating the therapeutic scenario, covering >50% of FDA approvals [3]. Companies are actively developing oral peptide

delivery technologies and/or have in their pipelines studies of oral peptide-targeting indications, such as cancer, diabetes, endometriosis, osteoporosis, bacterial infections, and others.

Nanoparticles

Nanoparticles (NPs), solid particles ranging between 1 and 100 nm in size, define delivery systems having colloidal properties when dispersed in an aqueous phase (e.g., liposomes, nanoemulsions, microemulsions, microclusters, colloidosomes, and biopolymers). In 2007, Gurny and colleagues described the improved solubility of low-soluble compounds formulated in NPs for oral administration by using polymers such as EUDRAGIT® L and S, and cellulose derivatives, such as hydroxypropyl methyl cellulose acetate succinate, hydroxypropyl methyl cellulose phthalate, cellulose acetate phthalate, and cellulose acetate trimellitate [4]. Enhanced bioavailability was also reported by Vol and colleagues, disclosing a pharmaceutical composition comprising silica NPs with a hydrophobic surface, branched with polysaccharides, and a biologically active protein or peptide embedded in an oil [5]. Following these findings, the Oshadi Drug Administration Ltd developed an oral carrier for insulin [5–7]. The Oshadi Icp, a combination of insulin, proinsulin, and C-peptide insulin, was shown to be safe and effective in lowering blood glucose [7]. Oshadi oral insulin is currently in Phase II clinical trials (Table 1). Whether the inorganic

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TABLE 1

Examples of orally administrated insulin strategies

Company	Name	Strategy	Comments	Status
Biocon Ltd	Insulin Tregopil	PE and PEGylation	Sodium caprate included as permeability enhancer in insulin prodrug (PEG-alkylated insulin) tablet	Phase I NCT04141423: T1DM; Phase II/III NCT03430856: T2DM
Diasome Pharmaceuticals Inc.	Oral HDV-Insulin	HDV	Insulin bound to HDV, the phospholipid bilayer of which has specific HTMs	Phase II NCT00814294 and NCT03096392: T2DM
Generex Biotechnology Corp.	Buccal insulin delivery; Oral-lyn™	Generex Oral-lyn™	Surfactants forming insulin-containing micelles are used as absorption enhancers; insulin delivered via Rapidmist™ device	Phase III NCT00668850: T1DM
Novo Nordisk Pharma AG	I338	GIPET®	Microemulsion systems based on medium-chain fatty acid glycerides formulated in enteric-coated tablets	Phase II NCT02470039: T2DM. Glycemic control and safety profile comparable to that of IGLar. Long-term effects of sodium caprate in intestine must be verified
	–	SOMA Milliposts	Self-orienting ingestible device comprising core of stainless steel and low-density polycaprolactone that autonomously deploys milliposts loaded with peptide into gastric epithelium	Preclinical studies. Milliposts of insulin successfully delivered showing blood glucose reduction. Stomach delivery makes dose delivery time more predictable. Device recovered from feces
	–	LUMI microneedle	Compressed spring driven by osmotic pump propelling drug-loaded microneedles into small intestine	Preclinical studies. No signs of inflammation observed. Device relies on gastric emptying to move from stomach to small intestine
Oramed Pharmaceuticals Inc.	ORMD-0801	POD™	EDTA, bile salts, peptidase inhibitors, and soyabean trypsin inhibitor incorporated with omega-3 fatty acids into oral enteric-coated formulation	Phase II NCT00867594: T1DM; lowered blood glucose and was well tolerated
Oshadi Drug Administration Ltd	Oshadi-lcp	Oshadi carrier NP	NP comprising silica core branched with polysaccharides, and oil combination of insulin, proinsulin, and C-peptide	Phase II NCT01973920: T1DM; plasma glucose lowering demonstrated along with good safety profile

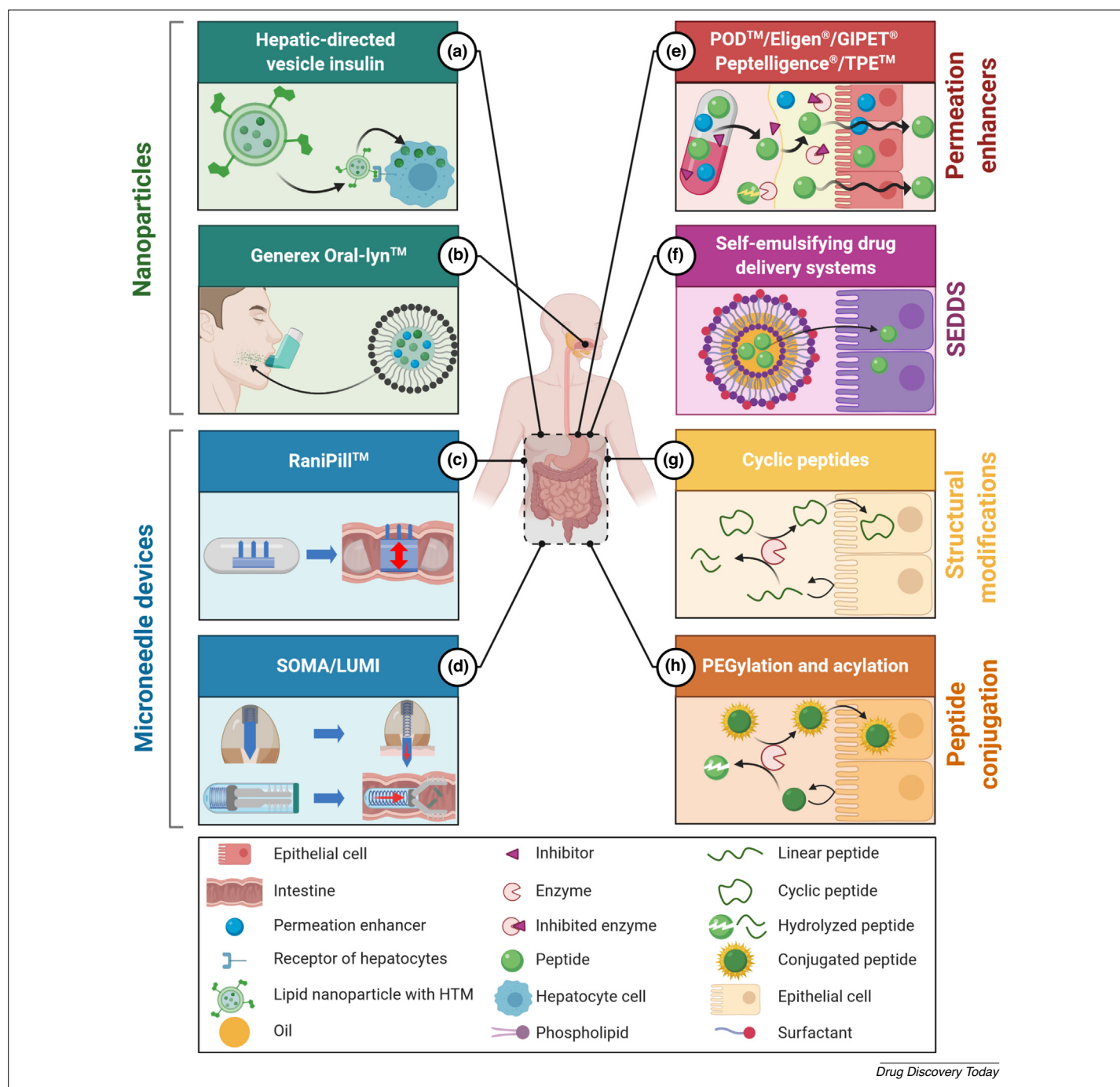
NPs will be cleared without body accumulation is still under investigation [8].

NPs are of interest because they promote a higher distribution of the drug at the intestinal epithelium [9]. However, the gastrointestinal (GI) tract mucus can reduce NP performance as delivery systems [10]. The mucus barrier, which has a protective function, affects the fate of oral therapeutic peptide NPs; thus, mucus-penetrating NPs have been developed. The addition of hydrophilic polymers, such as polyethylene glycol (PEG), displayed enhanced mucus penetration; insulin formulated in NPs coated with 2-hydroxypropyl methacrylamide polymer [11,12] showed improved epithelial uptake and reduced degradation compared with nonformulated insulin [13].

Diasome Pharmaceuticals Inc. developed a novel vesicle-containing insulin that is directly delivered to the liver. Hepatic-directed vesicle insulin (HDV-I) is a NP with a diameter <150 nm and a surface modified to be able to molecularly target hepatocytes (HTM) (Fig. 1). This allows uptake from the gut through the hepatic portal vein after oral administration and protects insulin from proteolytic degradation [14]. The oral formulation has no free insulin and all the insulin is bound to HDV and formulated as oral gel cap [15]. Diasome Pharmaceuticals Inc. performed clinical trials with HDV-I for subcutaneous (s.c.) and oral administration

[15]. Geho and colleagues observed that oral HDV-I was generally safe and well tolerated with no incidence of hypoglycemic episodes or serious adverse events, although with a lack of linear dose response [16]. Oral HDV-I for the treatment of T2DM is in clinical Phase IIb trials (Table 1), with Phase III trials planned for type 1 diabetes mellitus (T1DM) with injectable HDV-I [17].

Generex Oral-lyn™ is a liquid formulation of insulin delivered to the oral cavity through the RapidMist™ device (an aerosol-type device containing non-chlorofluorocarbon propellant, penetration enhancers, and stabilizers). The Oral-Lyn™ delivery system delivers insulin to the oral mucosa through micelles >7 nm in size, which, therefore, do not enter the respiratory system (Fig. 1). The surfactants used for the micelles containing insulin were shown to behave as permeation enhancers. The delivery device introduces a fine particle aerosol at high velocity into the oropharyngeal cavity for local transmucosal absorption [18]. In a study with patients with T1DM, Oral-lyn™ insulin was compared with insulin s.c. injections; Oral-lyn™ was shown to act more quickly compared with s.c. injections, suggesting that buccal insulin is more reflective of a normal insulin response to meal intake. Oral-lyn™ is in Phase III clinical trials for T1DM (Table 1) and was approved by the FDA in 2009 for treatment of patients not eligible for Phase III clinical trials under the Investigational New Drug (IND) program [19].

**FIGURE 1**

Assessment of techniques involved in peptide oral bioavailability improvement: **(a)** Hepatic-directed vesicle (HDV) insulin containing a hepatocyte-targeting molecule (HTM) on the nanoparticle surface. **(b)** Generex Oral-lyn™ comprises micelles enclosing insulin and permeation enhancers, applied with a propellant into the buccal cavity. Microneedle devices include **(c)** a RaniPill™, a robotic pill targeting peptides into the gastrointestinal (GI) tract, **(d)** SOMA, targeting the gastric epithelium and LUMI targeting the GI tract. **(e)** Permeation enhancers (POD™, Eligen®, GIPET®, Peptelligence®, and TPE™) are used in peptide formulations targeting the GI tract. **(f)** Self-emulsifying drug delivery systems (SEDDS) are mixtures of lipids, surfactants, and co-solvents that improve peptide bioavailability. **(g)** Cyclic peptides show improved stability from enzymatic degradation and enhanced membrane permeability. **(h)** Peptide conjugation, through PEGylation and acylation, results in synthetic peptides with improved physicochemical properties and better oral bioavailability. Figure created with BioRender.com.

Microneedle devices

Microneedle technology has been studied over the past two decades for several medical applications, from transdermal application, to vaccine delivery and targeting the cornea [20]. This

approach has been adapted for the delivery of peptides and proteins and represents a new concept to deliver molecules that notably have low oral bioavailability [21,22]. It is crucial to understand the interaction of such devices with the intestinal mucosa

because these approaches can be invasive, increasing the risk of mucosal perforation. Moreover, intestinal peristalsis is strongly patient dependent and, therefore, PK values can show considerable variation according to the patient's pathology.

The robotic pill or RaniPill™ is an enteric-coated capsule-like tablet. A hydroxypropyl methylcellulose capsule, enteric-coated with a polymer dissolving at a pH > 6.5, contains a balloon that, once swallowed, remains intact inside the stomach until it reaches

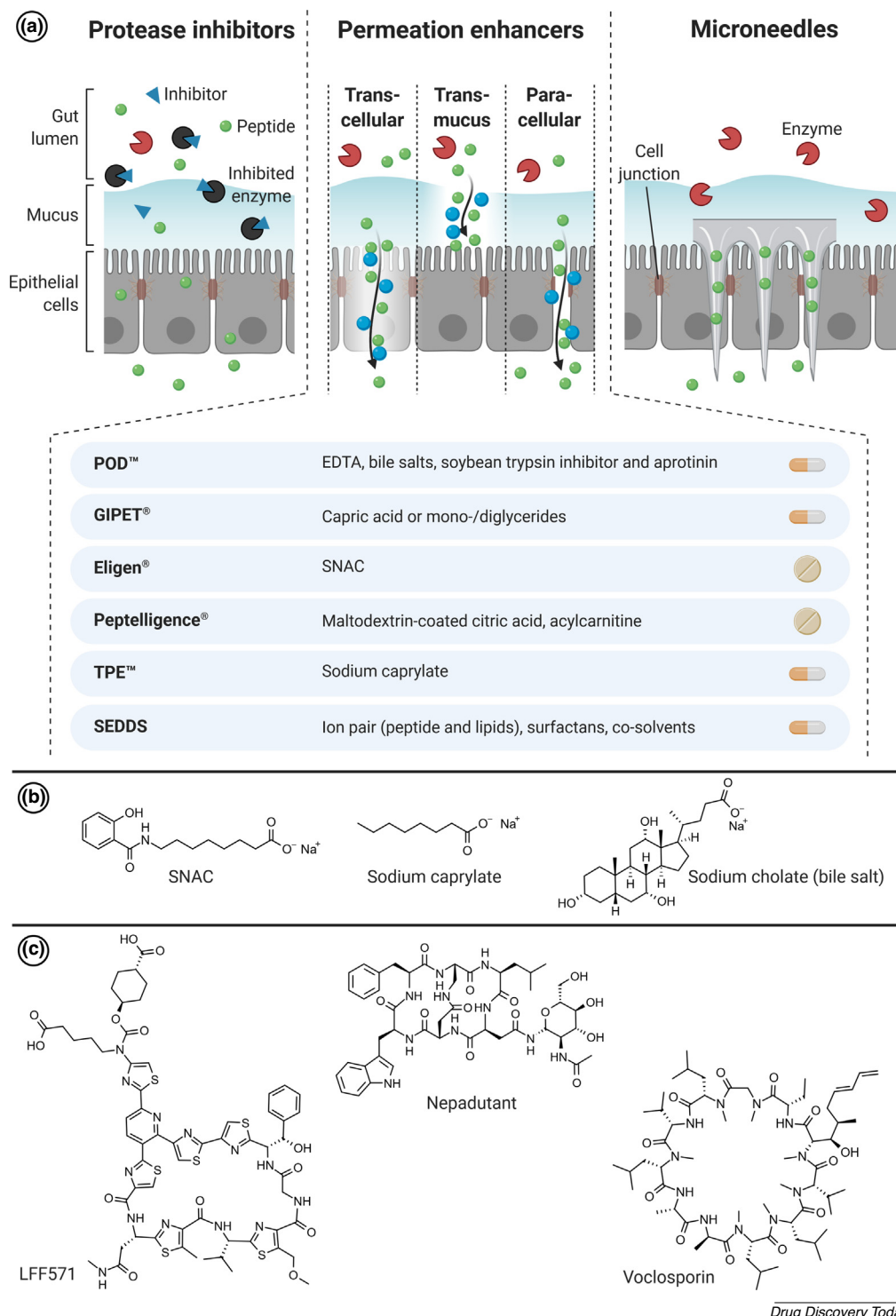


FIGURE 2

Schematic assessment of strategies involved in the oral delivery of peptides. **(a)** Protease inhibitors, permeation enhancers, and microneedles are effective for the oral delivery of peptides. **(b)** Permeation enhancers are successfully used for oral delivery, such as salcaprozate sodium (SNAC), bile salts, and sodium caprylate. They enable the passage of the molecule through the mucus layer, cell membranes, and tight junctions. **(c)** Chemical modification and cyclization are suitable tools to use to modulate the physicochemical properties of peptides and, thus, have been applied for the oral delivery of peptides. Figure created with [BioRender.com](https://www.biorender.com).

the jejunum. Here, the shell dissolves at the higher pH under the generation of carbon dioxide, inflating the balloon, which exposes dissolvable sucrose-based needles that pierce the intestinal wall and deliver the drug into the bloodstream [23] (Figs 1 and 2).

This cylindrical microneedle pill, measuring 2 cm in length and 1 cm in diameter, demonstrated its utility to deliver octreotide and is currently in Phase I trials (Table 1) [8]. Rani Therapeutics LLC has reported that the capsule has been safely tested in humans, reporting no adverse events [24]. Insulin delivery has been tested and preclinical studies have demonstrated that the relative oral availability of insulin was almost 100% compared with s.c. application [25]. However, the pill was manually inserted into the jejunal wall of the anesthetized animals and, to our knowledge, no other data are currently available; thus the performance of the pill needs to be evaluated in clinical studies.

Self-orienting millimeter-scale applicator (SOMA) is another oral delivery system inspired by the passive capability of the leopard tortoise to reorient (Fig. 1). This device was developed by Abramson and colleagues in 2019 as an ingestible device comprising a core of stainless steel and low-density polycaprolactone that autonomously deploys milliposts loaded with the peptide into the gastric epithelium [26]. Milliposts of insulin were successfully delivered in preclinical studies showing blood glucose reduction (Table 1). The device has been recovered in feces. Such direct stomach delivery rather than to the intestine would likely make the dose delivery time more predictable, given the variability in gastric emptying [27].

Luminal unfolding microneedle injectors (LUMI) are orally dosed devices created by the same group of Abramson, which insert drug-loaded microneedles into the small intestine wall (Table 1 and Fig. 1) [28]. This device is enclosed into a capsule (9 × 30 mm) coated with poly(methacrylic acid-co-ethyl acrylate), able to dissolve at a pH of ≥5.5; a compressed spring pushes the LUMI out of the capsule through an osmotic-controlled release delivery system called OROS®, which has been approved by the FDA [29]. In the GI tract, there is an established osmotic gradient, which draws water into the capsule. The released device has three degradable arms comprising biodegradable polyethylene oxide (PEO) and Soluplus® (a polyvinyl caprolactam-polyvinyl acetate polyethylene glycol graft copolymer), which expands the tissue wall and pushes a 1-mm patch with drug-loaded microneedles. The arms degrade within 24 h in vitro and in vivo. The insulin loaded device showed a systemic bioavailability higher than 10% compared to that observed with a s.c. injection [28].

The effect of prolonged use of these devices should be evaluated in long-term studies to understand the effects of microneedles on the intestinal mucosa. Moreover, to reach the intestine wall, the LUMI device relies on gastric emptying, which typically occurs in 1–4 h but can take up to 24 h in individuals with gastroparesis, as observed in patients with diabetes [27,30]. Thus, this factor could be cause of delay or uncertainty in the PK of this device.

Permeation enhancers

Oramed Pharmaceuticals Inc. developed Protein Oral Delivery (POD™). This is a system where different strategies coexist in the same formulation: encapsulation, permeation enhancers, a chelating agent, and a protease inhibitor. Oramed's technology is based on components aimed at providing protection during

passage through the GI tract and enhancing absorption. Ethylenediaminetetraacetate sodium (EDTA) and bile salts act as permeation enhancers. The capsule protects insulin from hydrolysis in the stomach, whereas protease inhibitors, such as soybean trypsin inhibitor and aprotinin, protect insulin from protease degradation, especially in the small intestine (Fig. 2) [31].

Insulin has been formulated with the Oramed technology, showing decreased postprandial glucose levels in patients with T1DM and in fasting patients with T2DM. Phase II clinical results from trials with patients with T1DM showed good tolerability. A completed Phase IIb trial also showed a significant lowering of mean night-time glucose levels in adults with T2DM (Table 1) [32].

The relevance of some surfactants and medium-chain fatty acids (MCFAs) as absorption enhancers is particularly relevant in such formations [33]. Fatty acids are nutrients released during the digestion of glycerides in the GI tract. The most extensively studied MCFAs are sodium caprate, a salt of capric acid, which comprises 2–3% of fatty acids in the milk fat fraction. Several compounds have been investigated for oral delivery of peptides: *N*-(5-chlorosalicyloyl)-8-aminocaprylic acid (5-CNAC) [34], 4-([4-chloro-2-hydroxybenzoyl]-amino) butanoic acid (4-CNAB) [35], and *N*-(8-[2-hydroxybenzoyl]-amino) caprylic acid, also known as salcaprozate sodium (SNAC) (Fig. 2).

Emisphere Technologies used SNAC as permeation enhancer for poorly intestinally permeable compounds (Fig. 1) [36]. SNAC, also known as Eligen®, is generally regarded as safe and was used to develop an oral vitamin B12. SNAC also resulted in improved oral bioavailability of insulin [35] and calcitonin [37], showing no toxicity and no impact on tight junctions [38]. Semaglutide, a glucagon-like peptide-1 (GLP-1) analog, has been co-formulated with the Eligen® technology as a tablet for oral administration. Semaglutide, a glucagon-like peptide-1 (GLP-1) analog, has been co-formulated with the Eligen® technology as a tablet for oral administration, showing to be well tolerated in subjects with varying degrees of renal impairments [39]. The Novo Nordisk clinical trial PIONEERS showed efficacy and safety in patients with T2DM and moderate renal impairment in a clinical trial Phase IIIa consistent with results for the GLP-1 receptor agonist (Table 2) [40]. The first oral GLP1 receptor agonist, semaglutide, using the Eligen® technology, was approved by the FDA in 2019 for the treatment of T2DM. Concerning the mechanism of action, many hypotheses have been provided. It is uncertain whether the SNAC permeation improvement results from membrane perturbation, solubility modulation, or tight junction opening, or whether it improves transcellular permeation (Fig. 2). However, the hypothesis of membrane perturbation was excluded by Novo Nordisk AG, who argued that SNAC creates a complex with semaglutide in the stomach, protecting the drug from pepsin and increasing the concentration-dependent flux of semaglutide through the gastric mucosa [41].

Merrion Pharmaceuticals Plc. developed GIPET® technology (Gastrointestinal Permeation Enhancement Technology). GIPET® comprises a sodium salt of capric acid, a soluble anionic surfactant, delivered in an enteric-coated solid dosage form. The first format of GIPET® (GIPET I) comprised enteric-coated tablets with a pH-sensitive coating (e.g., Eudragit®), a medium-chain fatty acid (e.g., C10) and a drug in selected ratios. The last modification of the technology (GIPET II) was an emulsion of mono- and

TABLE 2

Examples of orally administrated peptide drugs

Strategy	Comments	Name	Company	Status
Chemical modification cyclization	Generation of peptide bond between original N- and C- termini to overcome poor intestinal stability of linear peptides	Desmopressin, DDAVP®	Ferring Pharmaceuticals SA	Marketed. Indicated for diabetes insipidus, hemophilia A, von Willebrand disease, and high blood urea levels
		Voclosporin	Aurinia Pharmaceuticals Inc.	Phase III NCT03597464 AURORA2: active against lupus nephritis, uveitis
		Nepadutant	Menarini Pharma AG	Phase II NCT01258153NO-CRY: infant colic
		LFF571	Novartis Pharma AG	Phase II NCT01232595: <i>Clostridium difficile</i> infection
TPE™	Oily suspension formulated from sodium caprylate, PVP forming a hydrophilic powder suspended in lipophilic medium of glyceryl mono- and tricaprylate	Octreotide, Mycapps®	Chiasma Inc.	Phase III NCT03252353: acromegaly
Microneedle	In enteric-coated capsule, generation of carbon dioxide inflates a small balloon that pushes out sucrose-based needles	Octreotide	Rani Therapeutics LLC	Phase I NCT03798912: acromegaly
Peptelligence®	Enteric-coated tablet, the core of which comprises lyophilized peptide, maltodextrin-coated citric acid, and acylcarnitine	Salmon calcitonin, TBRIA™	Tarsa Therapeutics Inc.	Phase II NCT00959764 ORACAL: postmenopausal osteoporosis
		Leuprolide, Ovarest®	Enteris BioPharma Inc.	Phase II NCT02807363: endometriosis
		Difelikalin, KORSUVA™	Cara Therapeutics Inc.	Phase II rec. NCT04018027 KARE: pruritus, atopic dermatitis
Eligen®, Emisphere	SNAC as absorption enhancer in tablets with 3, 7, or 14 mg semaglutide	Semaglutide Rybelsus®	Novo Nordisk Pharma AG	Phase III NCT03015220 PIONEER 10: T2DM
SEDDS	Lipid-based microemulsion containing lipids, surfactant, and co-solvent	Cyclosporin A Sandimmune/Neoral®	Novartis Pharma AG	Marketed. Prevention of transplant rejection, treatment of patients with severe active, rheumatoid arthritis

diglyceride mixtures of C8 and C10 with the drug enclosed in an enteric-coated soft gel capsule (Figs 1 and 2). GIPET® has been used with alendronate and desmopressin to improve oral bioavailability in humans compared with unformulated controls [42]. Novo Nordisk insulin formulated with GIPET®, I338, is in Phase II clinical trials (Table 1). I338 was recently compared against subcutaneous insulin (iGlar), a well-established basal insulin treatment in patients with T2DM. The clinical trial Phase II study, completed in 2019, showed that oral insulin provided glycemic control not significantly different from that of iGlar over 8 weeks of once-daily treatment, and that the two products had a similar safety profile. The higher glycemic variability with I338 could increase the risk of hypoglycemia and the effects of the high concentration of sodium caprate in the intestine remain to be verified. Nevertheless, these results encourage the development of oral insulin products and strengthen the aspiration to make oral insulin available to patients with diabetes [43].

Peptelligence® technology is able to enhance paracellular transport in the intestine; the peptides are delivered in an enteric-coated tablet where the core comprises lyophilized peptide, maltodextrin-coated citric acid, and acylcarnitine [44,45]. Salmon calcitonin, TBRIA™, Leuprolide, Ovarest®, and Difelikalin, KORSUVA™ have been formulated with Peptelligence® and are in

Phase II trials. (Table 2). In the ORACAL trial, salmon calcitonin was evaluated for its efficacy and safety in women with postmenopausal osteoporosis: an improvement in the mineralization of spinal bone superior to that obtained with commercial nasal salmon calcitonin spray was observed, although adverse GI effects led to premature Phase III withdrawal [46].

Transient Permeability Enhancer, TPE™, developed by Chiasma Inc., and comprises a combination of excipients including sodium caprylate, in which the hydrophilic powder containing the active pharmaceutical ingredient is suspended, creating an oily suspension. (Fig. 1) [47]. Mycapps®, developed by Chiasma Inc., is an oral octreotide capsule formulated with TPE™ [48]. In vivo studies with octreotide, sodium caprylate, and fluorescein isothiocyanate (FITC)-labeled dextran showed permeation enhancement and reorganization of the tight junction proteins ZO-1 and claudin-3 [47]. The sodium caprylate excipient has a crucial role in permeation enhancement enabling transient and reversible paracellular tight junction passage of molecules (Fig. 2) [49], although the mechanism is not fully elucidated. The oral octreotide formulation was tested for efficacy and safety in a clinical trial Phase III in patients with acromegaly, showing to be effective and safe as an acromegaly monotherapy (Table 2). This study proved that daily oral administration of octreotide formulated with TPE™

resulted in plasma drug levels comparable to those with octreotide injection [50].

Self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems (SEDDS) are systems comprising mixtures of lipids, surfactants, and co-solvents. When dispersed in GI fluids, they form emulsions or microemulsions (Fig. 1). These systems received increased focus in recent years because of their ability to improve oral peptide delivery. They can help to overcome absorption barriers by providing protection against metabolism and improving permeation through the intestinal mucus layer; they can also be produced in a simple and cost-effective manner [51,52].

The only marketed peptide formulated with SEDDS is cyclosporin A (CsA; Sandimmune/Neoral® produced by Novartis AG), (Table 2). It is a microemulsion lipid-based formulation, leading to a bioavailability of 20–40% [53]. To improve absorption of the standard oral oil-based formulation of CsA, the lipid-based formulation Neoral® was developed. This new formulation is a microemulsion concentrate, which, in contact with GI fluids, forms a homogeneous, monophasic microemulsion, improving absorption and increasing the stability of the product [54].

Advanced SEDDS can include muco-inert compounds and cell-penetrating peptides contributing to create nanoemulsions with increased stability in GI fluids, improved mucus-permeating properties, and enhanced permeation (Fig. 2), resulting in the increased bioavailability of the incorporated peptides. A mucus-permeating SEDDS formulation for oral insulin delivery contains a hydrophobic ion pair (HIP) of insulin/dimyristoyl phosphatidylglycerol (INS/DMPG). More specifically, oil-surfactant-co-surfactant combinations were examined, and it was shown that the use of an increased amount of Labrafil® M1944CS (a long-chain triglyceride) in combination with Transcutol®P led to enhanced mucus permeability and high encapsulation efficiencies for insulin (i.e., up to 70%) [55]. In vivo studies with SEDDS with HIP of peptides have been carried out, displaying promising results; for example, exenatide was coupled with sodium docusate and incorporated into a SEDDS to increase mucus permeation [56].

Cyclic peptides

A successful strategy to overcome poor intestinal stability of linear peptides is peptide cyclization (Fig. 1). The beneficial properties of cyclic peptides are represented on the market by CsA, a natural cyclic peptide product formulated as a microemulsion (Neoral®), and desmopressin, an analog of the natural hormone vasopressin.

CsA is approved as an immunosuppressant and is available in two preparations, cyclosporine soft gelatin capsules and cyclosporine oral solution, which have been prescribed extensively as treatments following kidney, liver, and heart transplantations.

Desmopressin, a modified analog of vasopressin, was developed in tablet form for the treatment of diabetes insipidus during the 1980s and it is additionally administered for primary nocturnal enuresis. It shows greater antidiuretic activity than native vasopressin, and little vasoconstrictive activity. Desmopressin also has a prolonged antidiuretic action relative to native vasopressin, in part because of its resistance to degradation by vasopressinase (Table 2) [57].

Cyclic peptide candidates are also in clinical drug development. Voclosporin, a stabilized analogue of CsA (Aurinia Pharmaceuticals Inc.), has successfully completed Phase III clinical studies with an indication for the treatment of lupus nephritis (Table 2 and Fig. 2) [58]. Moreover, nepadutant is a glycosylated bicyclic hexapeptide antagonist of the receptor tachykinin NK2, designed by the Menarini Pharma AG group. Phase II clinical trials have been conducted on children with functional GI disorders (Fig. 2) [59]. Additionally, Phase II human trials of Novartis' LFF571 (Table 2 and Fig. 2), an oral semisynthetic thiopeptide antibiotic to treat *Clostridium difficile* infections, have been completed [60]. In these trials, the safety and efficacy of LFF571 were compared with those of vancomycin using adults with primary episodes or first recurrences of moderate *C. difficile* infection. Based on the results, the rate of clinical cure for LFF571 (90.6%) was higher compared with vancomycin (78.3%). In addition, the recurrence rates were lower for LFF571 compared with vancomycin [61]. However, the relevance of peptide cyclization as a generalized strategy for the improvement of oral availability is still under investigation. Nielsen *et al.* examined the physicochemical parameters and oral absorption of 125 cyclic peptides; although most of them were small, they showed poor oral bioavailability [62].

Peptide conjugation

A synthetic strategy that allows for the modulation of PK properties of therapeutic peptides is conjugation with moieties that extend half-life or improve solubility. Lipidization, where the peptide is linked with a fatty acid moiety, as well as PEGylation, are two successful approaches (Fig. 1). Insulin Tregopil (Biocon Lt.) is a novel PEGylated insulin analog modified for oral delivery. A single short-chain amphiphilic oligomer attached to the Lys-β29 residue of recombinant human insulin via an amide linkage increases the water solubility of the insulin analog. The alkylated PEG also provides improved stability against enzymatic degradation [63–65]. Insulin Tregopil has an additional sodium caprate excipient, a permeation enhancer, for further enhancement of absorption in the stomach [66]. An open-label Phase II/III study to evaluate the efficacy and safety of Insulin Tregopil in patients with T2DM was completed in February 2019, (Table 1). However, the results of this study have not yet been published.

Concluding remarks

There are many challenges to overcome when dealing with the oral delivery of peptides. The development of orally delivered peptides to replace injections remains a challenge, which would not only help improve the lives of patients, but also reduce healthcare costs worldwide. Orally delivered insulin is an example of this ultimate objective, providing undisputed therapeutic advantages for non-invasive chronic use. However, the improvement of life quality and the safety of patients must be the driving force of oral delivery peptide research. The solution to such a complex problem requires approaches from various disciplines, with formulation and chemical modification being vital.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors acknowledge financial support from the Swiss Innovation Agency (Innosuisse 34227.1 IP-LS; A.T.Z., D.P., and

R.R.), the Swiss National Science Foundation (SNSF CRSII5_183 481; F.M.G. and R.R.), and start-up funding AS-2019-2 from ZHAW Life Sciences und Facility Management (T.F. and R.R.).

References

- 1 Verified Market Research. *Peptide Therapeutics Market Size, Share, Trends & Forecast*, www.verifiedmarketresearch.com/product/peptide-therapeutics-market/ [Accessed January 18, 2021].
- 2 Allied Market Research. *Oral Proteins and Peptides Market Overview*, www.alliedmarketresearch.com/oral-proteins-peptides-market/ [Accessed January 18, 2021].
- 3 FDA. *Novel Drug Approvals for 2020*, www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2020/ [Accessed January 18, 2021].
- 4 Gurny, R. et al. Novartis. Nanoparticles for oral administration of pharmaceutical agents of low solubility. US20040018236A1.
- 5 Vol, A. and Gribova, O. Oshadi Drug Administration Ltd. Methods and compositions for oral administration of protein and peptide therapeutic agents. US9949924B2.
- 6 Vol, A. and Gribova, O. Oshadi Drug Administration Ltd. Methods and compositions for oral administration of protein and peptide therapeutic agents. US8936786B2.
- 7 Rachmiel, M. et al. (2019) OR14-1 pharmacodynamics, safety, tolerability, and efficacy of oral insulin formulation (Oshadi Icp) among young adults with type 1 diabetes: a summary of clinical studies phases I, Ib, and II. *J. Endocr. Soc.* 3. <http://dx.doi.org/10.1210/je.2019-OR14-1> Issue Supplement_1, April-May 2019, OR14-1
- 8 Brown, T.D. et al. (2020) Materials for oral delivery of proteins and peptides. *Nat. Rev. Mater.* 5, 127–148
- 9 Dumont, C. et al. (2018) Lipid-based nanosuspensions for oral delivery of peptides, a critical review. *Int. J. Pharm.* 541, 117–135
- 10 Ensign, L.M. et al. (2012) Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. *Adv. Drug Deliv. Rev.* 64, 557–570
- 11 Drucker, D.J. (2020) Advances in oral peptide therapeutics. *Nat. Rev. Drug Discov.* 19, 277–289
- 12 Shan, W. et al. (2015) Overcoming the diffusion barrier of mucus and absorption barrier of epithelium by self-assembled nanoparticles for oral delivery of insulin. *ACS Nano* 9, 2345–2356
- 13 Zheng, Y. et al. (2018) Multifunctional nanoparticles enable efficient oral delivery of biomacromolecules via improving payload stability and regulating the transcytosis pathway. *ACS Appl. Mater. Interfaces* 10, 34039–34049
- 14 Harris, E. (2017) An industry update: the latest news in therapeutic delivery. *Ther. Deliv.* 8, 939–946
- 15 Geho, W.B. et al. (2009) Hepatic-directed vesicle insulin: a review of formulation development and preclinical evaluation. *J. Diab. Sci. Technol.* 3, 1451–1459
- 16 Geho, W.B. et al. (2014) A single-blind, placebo-controlled, dose-ranging trial of oral hepatic-directed vesicle insulin add-on to oral antidiabetic treatment in patients with type 2 diabetes mellitus. *J. Diab. Sci. Technol.* 8, 551–559
- 17 Diasome. *Rethinking insulin*. www.diasome.com/breakthrough-hdv-technology/ [Accessed January 18, 2021].
- 18 Pozzilli, P. et al. (2010) Review of clinical trials: update on oral insulin spray formulation. *Diab. Obes. Metab.* 12, 91–96
- 19 Clinical Trials Arena. *Oral-Lyn - Oral Spray Formulation of Human Insulin for Types 1 and 2 Diabetes*. www.clinicaltrialsarena.com/projects/oral-lyn/ [Accessed January 18, 2021].
- 20 Kim, Y.-C. et al. (2012) Microneedles for drug and vaccine delivery. *Adv. Drug Deliv. Rev.* 64, 1547–1568
- 21 Fukushima, K. et al. (2011) Two-layered dissolving microneedles for percutaneous delivery of peptide/protein drugs in rats. *Pharm. Res.* 28, 7–21
- 22 Ito, Y. et al. (2010) Self-dissolving micropore array tips for percutaneous administration of insulin. *J. Mater. Sci. Mater. Med.* 21, 835–841
- 23 IEEE Spectrum. *Shot to the Gut: 'Robotic' Pill Sails through Human Safety Study*, <https://spectrum.ieee.org/the-human-os/biomedical/devices/shot-to-the-gut-robotic-pill-sails-through-human-safety-study/> [Accessed January 18, 2021].
- 24 Fierce Biotech. *Rani Therapeutics Completes First-in-Human Safety Study of its Robotic Biologic Pill*. <https://www.fiercebiotech.com/medtech/rani-therapeutics-completes-first-human-safety-study-its-robotic-biologic-pill/> [Accessed January 18, 2021].
- 25 Hashim, M. et al. (2019) Jejunal wall delivery of insulin via an ingestible capsule in anesthetized swine—a pharmacokinetic and pharmacodynamic study. *Pharmacol. Res. Perspect.* 7, e00522
- 26 Abramson, A. et al. (2019) An ingestible self-orienting system for oral delivery of macromolecules. *Science* 363, 611–615
- 27 Hellmig, S. et al. (2006) Gastric emptying time of fluids and solids in healthy subjects determined by 13C breath tests: influence of age, sex and body mass index. *J. Gastroenterol. Hepatol.* 21, 1832–1838
- 28 Abramson, A. et al. (2019) A luminal unfolding microneedle injector for oral delivery of macromolecules. *Nat. Med.* 25, 1512–1518
- 29 Bass, D.M. et al. (2002) Gastrointestinal safety of an extended-release, nondeformable, oral dosage form (OROS®)1. *Drug Saf.* 25, 1021–1033
- 30 Wang, Y.R. et al. (2008) Gastroparesis-related hospitalizations in the United States: trends, characteristics, and outcomes 1995–2004. *Am. J. Gastroenterol.* 103, 313–322
- 31 Kidron, M. Oramed Pharmaceuticals. Methods and compositions for oral administration of proteins. US20070087957A1.
- 32 Arbit, E. and Kidron, M. (2017) Oral insulin delivery in a physiologic context: review. *J. Diab. Sci. Technol.* 11, 825–832
- 33 Maher, S. et al. (2016) Intestinal permeation enhancers for oral peptide delivery. *Adv. Drug Deliv. Rev.* 106, 277–319
- 34 Karsdal, M.A. et al. (2015) Treatment of symptomatic knee osteoarthritis with oral salmon calcitonin: results from two phase 3 trials. *Osteoarthritis Cartilage* 23, 532–543
- 35 Kapitza, C. et al. (2010) Oral insulin: a comparison with subcutaneous regular human insulin in patients with type 2 diabetes. *Diab. Care* 33, 1288–1290
- 36 Leone-Bay, A. et al. (1995) N-Acylated alpha-amino acids as novel oral delivery agents for proteins. *J. Med. Chem.* 38, 4263–4269
- 37 Hamdy, R.C. and Daley, D.N. (2012) Oral calcitonin. *Int. J. Womens Health* 4, 471–479
- 38 Li, Y. et al. (2018) Impact of sodium N-8-(2-hydroxybenzoyl)amino]-caprylate on intestinal permeability for notoginsenoside R1 and salvianolic acids in Caco-2 cells transport and rat pharmacokinetics. *Molecules* 23, 2990
- 39 Granhall, C. et al. (2018) Pharmacokinetics, safety and tolerability of oral semaglutide in subjects with renal impairment. *Clin. Pharmacokinet.* 57, 1571–1580
- 40 Mosenzon, O. et al. (2019) Efficacy and safety of oral semaglutide in patients with type 2 diabetes and moderate renal impairment (PIONEER 5): a placebo-controlled, randomised, phase 3a trial. *Lancet Diab. Endocrinol.* 7, 515–527
- 41 Buckley, S.T. et al. (2018) Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist. *Sci. Transl. Med.* 10, eaar7047
- 42 Leonard, T.W. et al. (2006) Promoting absorption of drugs in humans using medium-chain fatty acid-based solid dosage forms: GIPET™. *Expert Opin. Drug Deliv.* 3, 685–692
- 43 Halberg, I.B. et al. (2019) Efficacy and safety of oral basal insulin versus subcutaneous insulin glargine in type 2 diabetes: a randomised, double-blind, phase 2 trial. *Lancet Diab. Endocrinol.* 7, 179–188
- 44 Drug Delivery Business. *Enteris BioPharma Touts more Phase II Data for Oral Endometriosis Therapy*. <https://www.drugdeliverybusiness.com/enteris-biopharma-touts-more-phase-ii-data-for-oral-endometriosis-therapy/> [Accessed January 18, 2021].
- 45 Drug Development and Delivery. *Drug delivery - Oral Delivery of Peptides by Peptelligence Technology*, <https://drug-dev.com/oral-delivery-of-peptides-by-peptelligence-technology/> [Accessed January 18, 2021].
- 46 Binkley, N. et al. (2012) A phase 3 trial of the efficacy and safety of oral recombinant calcitonin: The oral calcitonin in postmenopausal osteoporosis (ORACAL) trial. *J. Bone Miner. Res.* 27, 1821–1829
- 47 Tuvia, S. et al. (2014) A novel suspension formulation enhances intestinal absorption of macromolecules via transient and reversible transport mechanisms. *Pharm. Res.* 31, 2010–2021
- 48 Chiasma, Inc. *Corporate Profile*, <https://ir.chiasma.com/investor-overview/> [Accessed January 18, 2021].
- 49 Tuvia, S. et al. (2012) Oral octreotide absorption in human subjects: comparable pharmacokinetics to parenteral octreotide and effective growth hormone suppression. *J. Clin. Endocrinol. Metab.* 97, 2362–2369
- 50 Melmed, S. et al. (2015) Safety and efficacy of oral octreotide in acromegaly: results of a multicenter Phase III trial. *J. Clin. Endocrinol. Metab.* 100, 1699–1708
- 51 Leonaviciute, G. and Bernkop-Schnürch, A. (2015) Self-emulsifying drug delivery systems in oral (poly)peptide drug delivery. *Expert Opin. Drug Deliv.* 12, 1703–1716

- 52 Mahmood, A. and Bernkop-Schnürch, A. (2019) SEDDS: a game changing approach for the oral administration of hydrophilic macromolecular drugs. *Adv. Drug Deliv. Rev.* 142, 91–101
- 53 Czogalla, A. (2009) Oral cyclosporine A — the current picture of its liposomal and other delivery systems. *Cell. Mol. Biol. Lett.* 14, 139–152
- 54 Noble, S. and Markham, A. (1995) Cyclosporin: a review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral®). *Drugs* 50, 924–941
- 55 Karamanidou, T. *et al.* (2015) Effective incorporation of insulin in mucus permeating self-nanoemulsifying drug delivery systems. *Eur. J. Pharm. Biopharm.* 97, 223–229
- 56 Menzel, C. *et al.* (2018) *In vivo* evaluation of an oral self-emulsifying drug delivery system (SEDDS) for exenatide. *J. Control. Release* 277, 165–172
- 57 Qureshi, S. *et al.* (2014) Diabetes insipidus: celebrating a century of vasopressin therapy. *Endocrinology* 155, 4605–4621
- 58 Sin, F.E. and Isenberg, D. (2018) An evaluation of voclosporin for the treatment of lupus nephritis. *Expert Opin. Pharmacother.* 19, 1613–1621
- 59 Lecci, A. *et al.* (2001) Nepadutant pharmacokinetics and dose-effect relationships as tachykinin NK2 receptor antagonist are altered by intestinal inflammation in rodent models. *J. Pharmacol. Exp. Ther.* 299, 247–254
- 60 Jarrad, A.M. *et al.* (2015) *Clostridium difficile* drug pipeline: challenges in discovery and development of new agents. *J. Med. Chem.* 58, 5164–5185
- 61 Mullane, K. *et al.* (2015) Multicenter, randomized clinical trial to compare the safety and efficacy of LFF571 and vancomycin for *Clostridium difficile* infections. *Antimicrob. Agents Chemother.* 59, 1435–1440
- 62 Nielsen, D.S. *et al.* (2017) Orally absorbed cyclic peptides. *Chem. Rev.* 117, 8094–8128
- 63 Gregory, J.M. *et al.* (2019) Enterically delivered insulin tregopil exhibits rapid absorption characteristics and a pharmacodynamic effect similar to human insulin in conscious dogs. *Diab. Obes. Metab.* 21, 160–169
- 64 Gedawy, A. *et al.* (2018) Oral insulin delivery: existing barriers and current counter-strategies. *J. Pharm. Pharmacol.* 70, 197–213
- 65 Aguirre, T.A.S. *et al.* (2016) Current status of selected oral peptide technologies in advanced preclinical development and in clinical trials. *Adv. Drug Deliv. Rev.* 106, 223–241
- 66 Khedkar, A. *et al.* (2019) Impact of insulin tregopil and its permeation enhancer on pharmacokinetics of metformin in healthy volunteers: randomized, open-label, placebo-controlled, crossover study. *Clin. Transl. Sci.* 12, 276–282